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(54) METHODS AND COMPOSITIONS FOR ANALYZING NUCLEIC ACIDS BY MASS SPECTROMETRY

VERFAHREN UND VERBINDUNGEN ZUR ANALYSIERUNG VON NUKLEINSAEUREN DURCH MASSENSPEKTROMETRIE

PROCEDES ET COMPOSITIONS POUR L'ANALYSE DE MOLECULES D'ACIDES NUCLEIQUES AU MOYEN DE SPECTROMETRIE DE MASSE

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(73) Proprietor: **Qiagen Genomics, Inc.**
Bothell, Washington 98021 (US)

(72) Inventors:
- **VAN NESS, Jeffrey**
  Seattle, WA 98125 (US)
- **TABONE, John, C.**
  Bothell, WA 98011 (US)
- **HOWBERT, Jeffry**
  Bellevue, WA 98005 (US)
- **MULLIGAN, John, T.**
  Seattle, WA 98105 (US)

(74) Representative: **Gowshall, Jonathan Vallance**
FORRESTER & BOEHMERT
Pettenkoferstrasse 20-22
80336 München (DE)

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EP 0 990 047 B1

Claims

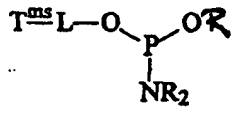
1. A compound of the formula:

$$T^{ms}\text{-}L\text{-}X$$

wherein,

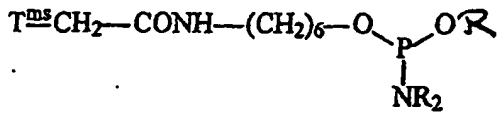
T^{ms} is an organic group detectable by mass spectrometry, comprising carbon, at least one of hydrogen and fluoride, and optional atoms selected from oxygen, nitrogen, sulfur, phosphorus and iodine;
L is an organic group which allows a unique T^{ms}-containing moiety to be cleaved from the remainder of the compound, wherein the T^{ms}-containing moiety comprises a functional group which supports a single ionized charge state when the compound is subjected to mass spectrometry and is tertiary amine, quaternary amine or organic acid;
X is a functional group selected from phosphoramidite and H-phosphonate.

2. The compound of claim 1 wherein X is a phosphoramidite group such that T^{ms}-L-X has the structure



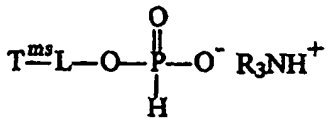
wherein R is an alkyl group or a substituted alkyl group having one or more substituents selected from halogen and cyano, and the two R groups of NR_2 may be bonded together to form a cycloalkyl group.

3. The compound of claim 2 wherein X is a phosphoramidite group such that T^{ms}-L-X has the structure



and OR is OCH_2CH_2CN while NR_2 is N(iso-propyl)$_2$.

4. The compound of claim 1 wherein X has an H-phosphonate group such that T^{ms}-L-X has the structure



wherein R is a C_1-C_6alkyl group.

5. A compound according to claims 1-4 wherein T^{ms} has a mass of from 15 to 10,000 daltons and a molecular formula of $C_{1\text{-}500}N_{0\text{-}100}O_{0\text{-}100}S_{0\text{-}10}P_{0\text{-}10}H_{\alpha}F_{\beta}I_{\delta}$ wherein the sum of α, β and δ is sufficient to satisfy the otherwise unsatisfied valencies of the C, N and O atoms.

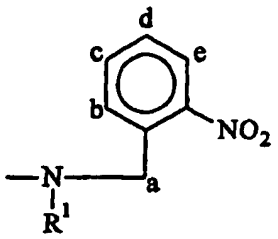
6. A compound according to claims 1-4 wherein T^{ms} and L are bonded together through a functional group selected from amide, ester, ether, amine, sulfide, thioester, disulfide, thioether, urea, thiourea, carbamate, thiocarbamate, Schiff base, reduced Schiff base, imine, oxime, hydrazone, phosphate, phosphpnate, phosphoramide, phosphona-

mide, sulfonate, sulfonamide or carbon-carbon bond.

7. A compound according to claims 1-4 wherein L is selected from L^{hv}, L^{acid}, L^{base}, $L^{[O]}$, $L^{[R]}$, L^{enz}, L^{elc}, L^{Δ} and L^{ss}, where actinic radiation, acid, base, oxidation, reduction, enzyme, electrochemical, thermal and thiol exchange, respectively, cause the T^{ms}-containing moiety to be cleaved from the remainder of the molecule.

8. A compound according to claims 1-4 wherein L^{hv} has the formula L^1-L^2-L^3, wherein L^2 is a molecular fragment that absorbs actinic radiation to promote the cleavage of T^{ms} from X, and L^1 and L^3 are independently a direct bond or an organic moiety, where L^1 separates L^2 from T^{ms} and L^3 separates L^2 from X, and neither L^1 nor L^3 undergo bond cleavage when L^2 absorbs the actinic radiation.

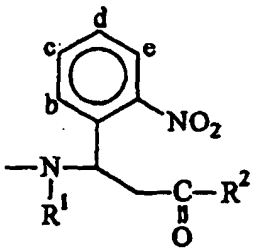
9. A compound according to claim 8 wherein -L^2-L^3 has the formula:



with one carbon atom at positions a, b, c, d or e being substituted with -L^3-X and optionally one or more of positions b, c, d or e being substituted with alkyl, alkoxy, fluoride, chloride, hydroxyl, carboxylate or amide; and R^1 is hydrogen or hydrocarbyl.

10. A compound according to claim 8 wherein L^3 is selected from a direct bond, a hydrocarbylene, -O-hydrocarbylene, and hydrocarbylene-(O-hydrocarbylene)$_n$-H, and n is an integer ranging from 1 to 10.

11. A compound according to claims 1-4 wherein -L-X has the formula:



wherein one or more of positions b, c, d or e is substituted with hydrogen, alkyl, alkoxy, fluoride, chloride, hydroxyl, carboxylate or amide; R^1 is hydrogen or hydrocarbyl, and R^2 terminates in an "X" group.

12. A compound according to claims 1-4 wherein T^{ms} has the formula:

$$T^2\text{-}(J\text{-}T^3\text{-})_n\text{-}$$

T^2 is an organic moiety formed from carbon and one or more of hydrogen, fluoride, iodide, oxygen, nitrogen, sulfur and phosphorous, having a mass of 15 to 500 daltons;
T^3 is an organic moiety formed from carbon and one or more of hydrogen, fluoride, iodide, oxygen, nitrogen, sulfur and phosphorous, having a mass of 50 to 1000 daltons;
J is a direct bond or a functional group selected from amide, ester, amine, sulfide, ether, thioester, disulfide, thioether, urea, thiourea, carbamate, thiocarbamate, Schiff base, reduced Schiff base, imine, oxime, hydra-

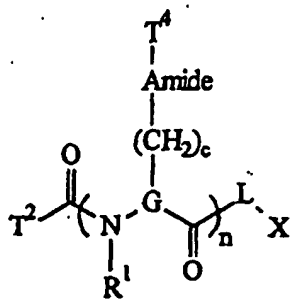
zone, phosphate, phosphonate, phosphoramide, phosphonamide, sulfonate, sulfonamide or carbon-carbon bond; and

n is an integer ranging from 1 to 50, and when n is greater than 1, each T^3 and J is independently selected.

13. A compound according to claim 12 wherein T^2 is selected from hydrocarbyl, hydrocarbyl-O-hydrocarbylene, hydrocarbyl-S-hydrocarbylene, hydrocarbyl-NH-hydrocarbylene, hydrocarbyl-amide-hydrocarbylene, N-(hydrocarbyl)hydrocarbylene, N,N-di(hydrocarbyl)hydrocarbylene, hydrocarbylacyl-hydrocarbylene, heterocyclylhydrocarbyl wherein the heteroatom(s) are selected from oxygen, nitrogen, sulfur and phosphorous, substituted heterocyclylhydrocarbyl wherein the heteroatom(s) are selected from oxygen, nitrogen, sulfur and phosphorous and the substituents are selected from hydrocarbyl, hydrocarbyl-O-hydrocarbylene, hydrocarbyl-NH-hydrocarbylene, hydrocarbyl-S-hydrocarbylene, N-(hydrocarbyl)hydrocarbylene, N,N-di(hydrocarbyl)hydrocarbylene and hydrocarbylacyl-hydrocarbylene, as well as derivatives of any of the foregoing wherein one or more hydrogens is replaced with an equal number of fluorides.

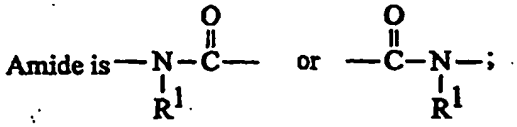
14. A compound according to claim 12 wherein T^3 has the formula - $G(R^2)$-, G is C_{1-6} alkylene having a single R^2 substituent, and R^2 is selected from alkyl, alkenyl, alkynyl, cycloalkyl, aryl-fused cycloalkyl, cycloalkenyl, aryl, aralkyl, aryl-substituted alkenyl or alkynyl, cycloalkyl-substituted alkyl, cycloalkenyl-substituted cycloalkyl, biaryl, alkoxy, alkenoxy, alkynoxy, aralkoxy, aryl-substituted alkenoxy or alkynoxy, alkylamino, alkenylamino or alkynylamino, aryl-substituted alkylamino, aryl-substituted alkenylamino or alkynylamino, aryloxy, arylamino, N-alkylurea-substituted alkyl, N-arylurea-substituted alkyl, alkylcarbonylamino-substituted alkyl, aminocarbonyl-substituted alkyl, heterocyclyl, heterocyclyl-substituted alkyl, heterocyclyl-substituted amino, carboxyalkyl substituted aralkyl, oxocarbocyclyl-fused aryl and heterocyclylalkyl; cycloalkenyl, aryl-substituted alkyl and, aralkyl, hydroxy-substituted alkyl, alkoxy-substituted alkyl, aralkoxy-substituted alkyl, alkoxy-substituted alkyl, aralkoxy-substituted alkyl, amino-substituted alkyl, (aryl-substituted alkyloxycarbonylamino)-substituted alkyl, thiol-substituted alkyl, alkylsulfonyl-substituted alkyl, (hydroxy-substituted alkylthio)-substituted alkyl, thioalkoxy-substituted alkyl, hydrocarbylacylamino-substituted alkyl, heterocyclylacylamino-substituted alkyl, hydrocarbyl-substituted-heterocyclylacylamino-substituted alkyl, alkylsulfonylamino-substituted alkyl, arylsulfonylamino-substituted alkyl, morpholino-alkyl, thiomorpholino-alkyl, morpholino carbonyl-substituted alkyl, thiomorpholinocarbonyl-substituted alkyl, [N-(alkyl, alkenyl or alkynyl)- or N,N-[dialkyl, dialkenyl, dialkynyl or (alkyl, alkenyl)-amino]carbonyl-substituted alkyl, heterocyclylaminocarbonyl, heterocylylalkyleneaminocarbonyl, heterocyclylaminocarbonyl-substituted alkyl, heterocylylalkyleneaminocarbonyl-substituted alkyl, N,N-[dialkyl]alkyleneaminocarbonyl, N,N-[dialkyl]alkyleneaminocarbonyl-substituted alkyl, alkyl-substituted heterocyclylcarbonyl, alkyl-substituted heterocyclylcarbonyl-alkyl, carboxyl-substituted alkyl, dialkylamino-substituted acylaminoalkyl and amino acid side chains selected from arginine, asparagine, glutamine, S-methyl cysteine, methionine and corresponding sulfoxide and sulfone derivatives thereof, glycine, leucine, isoleucine, allo-isoleucine, tert-leucine, norleucine, phenylalanine, tyrosine, tryptophan, proline, alanine, ornithine, histidine, glutamine, valine, threonine, serine, aspartic acid, beta-cyanoalanine, and allothreonine; alynyl and heterocyclylcarbonyl, aminocarbonyl, amido, mono- or dialkylaminocarbonyl, mono- or diarylaminocarbonyl, alkylarylaminocarbonyl, diarylaminocarbonyl, mono- or diacylaminocarbonyl, aromatic or aliphatic acyl, alkyl optionally substituted by substituents selected from amino, carboxy, hydroxy, mercapto, mono- or dialkylamino, mono- or diarylamino, alkylarylamino, diarylamino, mono- or diacylamino, alkoxy, alkenoxy, aryloxy, thioalkoxy, thioalkenoxy, thioalkynoxy, thioaryloxy and heterocyclyl.

15. A compound according to claim 12 having the formula:



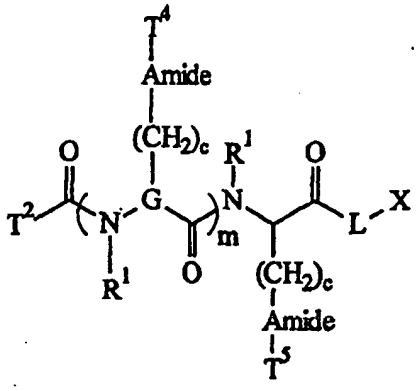
wherein

G is $(CH_2)_{1-6}$ wherein a hydrogen on one and only one of the CH_2 groups is replaced with-$(CH_2)_c$-Amide-T^4; T^2 and T^4 are organic moieties of the formula $C_{1-25}N_{0-9}O_{0-9}H_\alpha F_\beta$ wherein the sum of α and β is sufficient to satisfy the otherwise unsatisfied valencies of the C, N, and O atoms;



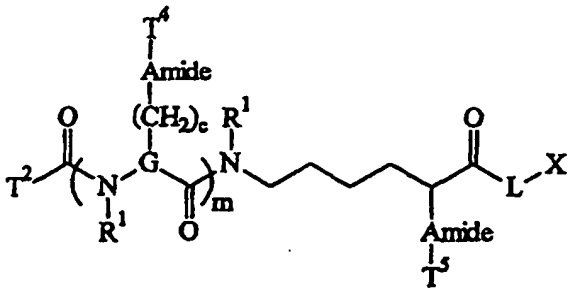
R^1 is hydrogen or C_{1-10} alkyl;
c is an integer ranging from 0 to 4;
X is defined according to claim 1; and
n is an integer ranging from 1 to 50 such that when n is greater than 1, G, c, Amide, R^1 and T^4 are independently selected.

16. A compound according to claim 15 having the formula:



wherein T^5 is an organic moiety of the formula $C_{1-25}N_{0-9}O_{0-9}H_\alpha F_\beta$ wherein the sum of α and β is sufficient to satisfy the otherwise unsatisfied valencies of the C, N, and O atoms; and T^5 includes a tertiary or quaternary amine or an organic acid; and m is an integer ranging from 0-49.

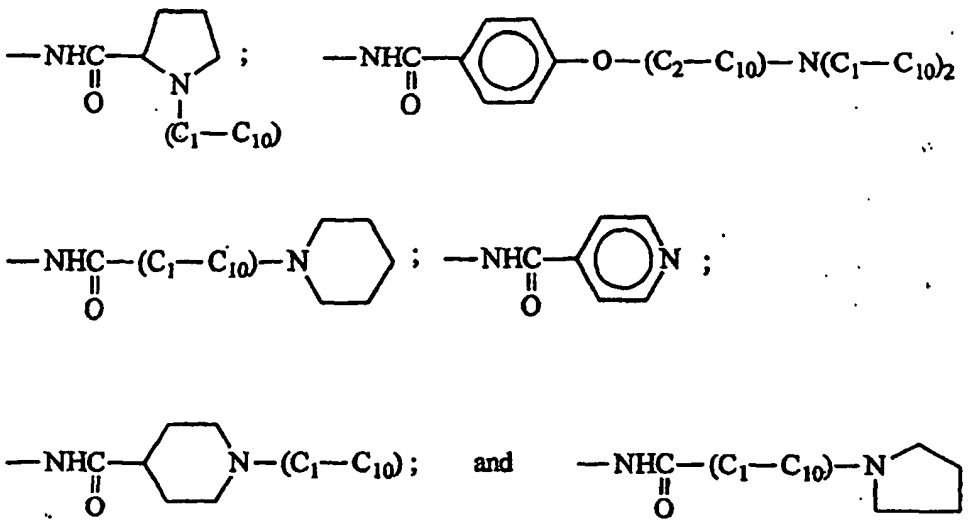
17. A compound according to claim 15 having the formula:



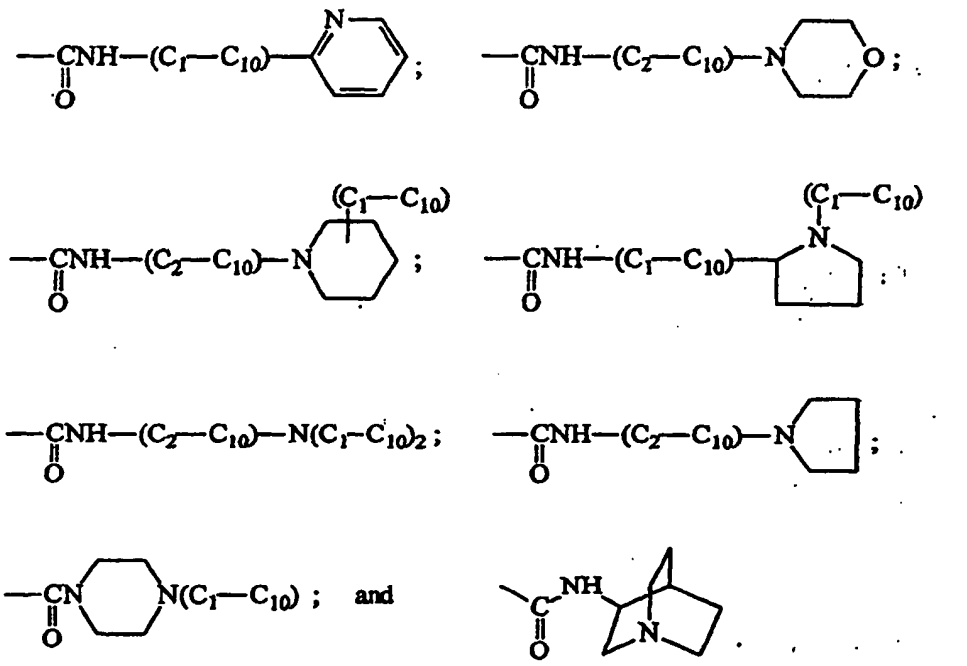
wherein T^5 is an organic moiety of the formula $C_{1-25}N_{0-9}O_{0-9}H_\alpha F_\beta$ wherein the sum of α and β is sufficient to satisfy the otherwise unsatisfied valencies of the C, N, and O atoms; and T^5 includes a tertiary or quaternary

amine or an organic acid; and m is an integer ranging from 0-49.

18. A compound according to any one of claims 16 and 17 wherein -Amide-T^5 is selected from:



19. A compound according to any of claims 16 and 17 wherein -Amide-T^5 is selected from:



20. A compound according to any one of claims 14-16 wherein T^2 has the structure which results when one of the following organic acids is condensed with an amine group to form T^2-C(=O)-N(R^1)-: Formic acid, Acetic acid, Propiolic acid, Proplonic acid, Fluoroacetic acid, 2-Butynoic acid, Cyclopropanecarboxylic acid, Butyric acid, Methoxyacetic acid, Difluoroacetic acid, 4-Pentynoic acid, Cyclobutanecarboxylic acid, 3,3-Dimethylacrylic acid, Valeric

acid, N,N-Dimethylglycine, N-Formyl-Gly-OH, Ethoxyacetic acid, (Methylthio)acetic acid, Pyrrole-2-carboxylic acid, 3-Furoic acid, Isoxazole-5-carboxylic acid, trans-3-Hexenoic acid, Trifluoroacetic acid, Hexanoic acid, Ac-Gly-OH, 2-Hydroxy-2-methylbutyric acid, Benzoic acid, Nicotinic acid, 2-Pyrazinecarboxylic acid, 1-Methyl-2-pyrrolecarboxylic acid, 2-Cyclopentene-1-acetic acid, Cyclopentylacetic acid, (S)-(-)-2-Pyrrolidone-5-carboxylic acid, N-Methyl-L-proline, Heptanoic acid, Ac-b-Ala-OH, 2-Ethyl-2-hydroxybutyric acid, 2-(2-Methoxyethoxy)acetic acid, p-Toluic acid, 6-Methylnicotinic acid, 5-Methyl-2-pyrazinecarboxylic acid, 2,5-Dimethylpyrrole-3-carboxylic acid, 4-Fluorobenzoic acid, 3,5-Dimethylisoxazole-4-carboxylic acid, 3-Cyclopentylpropionic acid, Octanoic acid, N,N-Dimethylsuccinamic acid, Phenylpropiolic acid, Cinnamic acid, 4-Ethylbenzoic acid, p-Anisic acid, 1,2,5-Trimethylpyrrole-3-carboxylic acid, 3-Fluoro-4-methylbenzoic acid, Ac-DL-Propargylglycine, 3-(Trifluoromethyl)butyric acid, 1-Piperidinepropionic acid, N-Acetylproline, 3,5-Difluorobenzoic acid, Ac-L-Val-OH, Indole-2-carboxylic acid, 2-Benzofurancarboxylic acid, Benzotriazole-5-carboxylic acid, 4-n-Propylbenzoic acid, 3-Dimethylaminobenzoic acid, 4-Ethoxybenzoic acid, 4-(Methylthio)benzoic acid, N-(2-Furoyl)glycine, 2-(Methylthio)nicotinic acid, 3-Fluoro-4-methoxybenzoic acid, Tfa-Gly-OH, 2-Napthoic acid, Quinaldic acid, Ac-L-Ile-OH, 3-Methylindene-2-carboxylic acid, 2-Quinoxalinecarboxylic acid, 1-Methylindole-2-carboxylic acid, 2,3,6-Trifluorobenzoic acid, N-Formyl-L-Met-OH, 2-[2-(2-Methoxyethoxy)ethoxy]acetic acid, 4-n-Butylbenzoic acid, N-Benzoylglycine, 5-Fluoroindole-2-carboxylic acid, 4-n-Propoxybenzoic acid, 4-Acetyl-3,5-dimethyl-2-pyrrolecarboxylic acid, 3,5-Dimethoxybenzoic acid, 2,6-Dimethoxynicotinic acid, Cyclohexanepentanoic acid, 2-Naphthylacetic acid, 4-(1H-Pyrrol-1-yl)benzoic acid, Indole-3-propionic acid, m-Trifluoromethylbenzoic acid, 5-Methoxyindole-2-carboxylic acid, 4-Pentylbenzoic acid, Bz-b-Ala-OH, 4-Diethylaminobenzoic acid, 4-n-Butoxybenzoic acid, 3-Methyl-5-CF3-isoxazole-4-carboxylic acid, (3,4-Dimethoxyphenyl)acetic acid, 4-Biphenylcarboxylic acid, Pivaloyl-Pro-OH, Octanoyl-Gly-OH, (2-Naphthoxy)acetic acid, Indole-3-butyric acid, 4-(Trifluoromethyl)phenylacetic acid, 5-Methoxyindole-3-acetic acid, 4-(Trifluoromethoxy)benzoic acid, Ac-L-Phe-OH, 4-Pentyloxybenzoic acid, Z-Gly-OH, 4-Carboxy-N-(fur-2-ylmethyl)pyrrolidin-2-one, 3,4-Diethoxybenzoic acid, 2,4-Dimethyl-5-CO_2Et-pyrrole-3-carboxylic acid, N-(2-Fluorophenyl)succinamic acid, 3,4,5-Trimethoxybenzoic acid, N-Phenylanthranilic acid, 3-Phenoxybenzoic acid, Nonanoyl-Gly-OH, 2-Phenoxypyridine-3-carboxylic acid, 2,5-Dimethyl-1-phenylpyrrole-3-carboxylic acid, trans-4-(Trifluoromethyl)cinnamic acid, (5-Methyl-2-phenyloxazol-4-yl)acetic acid, 4-(2-Cyclohexenyloxy)benzoic acid, 5-Methoxy-2-methylindole-3-acetic acid, trans-4-Cotininecarboxylic acid, Bz-5-Aminovaleric acid, 4-Hexyloxybenzoic acid, N-(3-Methoxyphenyl)succinamic acid, Z-Sar-OH, 4-(3,4-Dimethoxyphenyl)butyric acid, Ac-o-Fluoro-DL-Phe-OH, N-(4-Fluorophenyl)glutaramic acid, 4'-Ethyl-4-biphenylcarboxylic acid, 1,2,3,4-Tetrahydroacridinecarboxylic acid, 3-Phenoxyphenylacetic acid, N-(2,4-Difluorophenyl)succinamic acid, N-Decanoyl-Gly-OH, (+)-6-Methoxy-a-methyl-2-naphthaleneacetic acid, 3-(Trifluoromethoxy)cinnamic acid, N-Formyl-DL-Trp-OH, (R)-(+)-a-Methoxy-a-(trifluoromethyl)phenylacetic acid, Bz-DL-Leu-OH, 4-(Trifluoromethoxy)phenoxyacetic acid, 4-Heptyloxybenzoic acid, 2,3,4-Trimethoxycinnamic acid, 2,6-Dimethoxybenzoyl-Gly-OH, 3-(3,4,5-Trimethoxyphenyl)propionic acid, 2,3,4,5,6-Pentafluorophenoxyacetic acid, N-(2,4-Difluorophenyl)glutaramic acid, N-Undecanoyl-Gly-OH, 2-(4-Fluorobenzoyl)benzoic acid, 5-Trifluoromethoxyindole-2-carboxylic acid, N-(2,4-Difluorophenyl)diglycolamic acid, Ac-L-Trp-OH, Tfa-L-Phenylglycine-OH, 3-Iodobenzoic acid, 3-(4-n-Pentylbenzoyl)propionic acid, 2-Phenyl-4-quinolinecarboxylic acid, 4-Octyloxybenzoic acid, Bz-L-Met-OH, 3,4,5-Triethoxybenzoic acid, N-Lauroyl-Gly-OH, 3,5-Bis(trifluoromethyl)benzoic acid, Ac-5-Methyl-DL-Trp-OH, 2-Iodophenylacetic acid, 3-Iodo-4-methylbenzoic acid, 3-(4-n-Hexylbenzoyl)propionic acid, N-Hexanoyl-L-Phe-OH, 4-Nonyloxybenzoic acid, 4'-(Trifluoromethyl)-2-biphenylcarboxylic acid, Bz-L-Phe-OH, N-Tridecanoyl-Gly-OH, 3,5-Bis(trifluoromethyl)phenylacetic acid, 3-(4-n-Heptylbenzoyl)propionic acid, N-Hepytanoyl-L-Phe-OH, 4-Decyloxybenzoic acid, N-(α,α,α-trifluoro-m-tolyl) anthranilic acid, Niflumic acid, 4-(2-Hydroxyhexafluoroisopropyl)benzoic acid, N-Myristoyl-Gly-OH, 3-(4-n-Octylbenzoyl)propionic acid, N-Octanoyl-L-Phe-OH, 4-Undecyloxybenzoic acid, 3-(3,4,5-Trimethoxyphenyl)propionyl-Gly-OH, 8-Iodonaphthoic acid, N-Pentadecanoyl-Gly-OH, 4-Dodecyloxybenzoic acid, N-Palmitoyl-Gly-OH, and N-Stearoyl-Gly-OH.

21. A method for determining the presence of a single nucleotide polymorphism in a nucleic acid target comprising:

a) amplifying a sequence of a nucleic acid target containing a single nucleotide polymorphism;
b) generating a single strand form of the target;
c) combining a tagged nucleic acid probe with the amplified target nucleic acid molecules under conditions and for a time sufficient to permit hybridization of said tagged nucleic acid probe to complementary amplified selected target nucleic acid molecules, wherein said tag is correlative with a particular single nucleotide polymorphism and is detectable by spectrometry or potentiometry;
d) separating unhybridized tagged probe from hybridized tagged probe by a sizing methodology;
e) cleaving said tag from said probe; and
f) detecting said tag by spectrometry or potentiometry, and determining the presence of said single nucleotide polymorphism,

wherein the tagged nucleic acid probe has the structure

$$T^{ms}\text{-}L\text{-}X'$$

wherein,

T^{ms} is an organic group detectable by mass spectrometry, comprising carbon, at least one of hydrogen and fluoride, and optional atoms selected from oxygen, nitrogen, sulfur, phosphorus and iodine;
L is an organic group which allows a unique T^{ms}-containing moiety to be cleaved from the remainder of the compound, wherein the T^{ms}-containing moiety, comprises a functional group which supports a single ionized charge state when the compound is subjected to mass spectrometry and is tertiary amine, quaternary amine or organic acid; and
X' is nucleic acid.

22. A method for determining the presence of a single nucleotide polymorphism in a nucleic acid target comprising:

a) amplifying a sequence of a nucleic acid target containing a single nucleotide polymorphism;
b) combining a tagged nucleic acid primer with the amplified target nucleic acid molecules under conditions and for a time sufficient to permit annealing of said tagged nucleic acid primer to complementary amplified selected target nucleic acid molecules, wherein the oligonucleotide primer has a 3'-most base complementary to the wildtype sequence or the single nucleotide polymorphism, wherein said tag is correlative with a particular single nucleotide polymorphism and is detectable by spectrometry or potentiometry;
c) extending the primer wherein a complementary strand to the target is synthesized when the 3'-most baseof the primer is complementary to the target;
d) separating unextended tagged primer from extended tagged primer by a sizing methodology;
e) cleaving said tag from said primers or extended primers; and
f) detecting said tag by spectrometry or potentiometry, and determining therefrom the presence of said single nucleotide polymorphis,

wherein the tagged nucleic acid primer has the structure

$$T^{ms}\text{-}L\text{-}X'$$

wherein,

T^{ms} is an organic group detectable by mass spectrometry, comprising carbon, at least one of hydrogen and fluoride, and optional atoms selected from oxygen, nitrogen, sulfur, phosphorus and iodine;
L is an organic group which allows a unique T^{ms}-containing moiety to be cleaved from the remainder of the compound, wherein the T^{ms}-containing moiety comprises a functional group which supports a single ionized charge state when the compound is subjected to mass spectrometry and is tertiary amine, quaternary amine or organic acid; and
X' is nucleic acid.

23. A method for determining the quantity of a specific mRNA molecule in a nucleic acid population comprising:

a) converting an RNA population into a cDNA population;
b) adding a single strand nucleic acid (internal standard) containing a plurality of single nucleotide polymorphisms, that is otherwise identical to said cDNA target;
c) amplifying a specific sequence of said cDNA target;
d) coamplifying the internal standard, wherein said internal standard is the same length as the cDNA amplicon;
e) generating a single strand form of the target;
f) combining a set of tagged nucleic acid probes with the amplified target cDNA and amplified internal standard under conditions and for a time sufficient to permit hybridization of said tagged nucleic acid probe to complementary selected target cDNA and internal standard sequences, wherein said tag is correlative with a particular cDNA sequence and a second tag is correlative with the internal standard, and is detectable by spectrometry or potentiometry;

g) separating unhybridized tagged probe from hybridized tagged probe by a sizing methodology;
h) cleaving said tag from said probes;
i) detecting said tags by spectrometry or potentiometry; and
j) taking the ratio of tag correlated to cDNA to tag correlated with the internal standard, and determining therefrom the quantity of said cDNA, thereby determining the quantity of the specific mRNA in a nucleic acid population,

wherein the tagged nucleic acid probe has the structure

$$T^{ms}\text{-}L\text{-}X'$$

wherein,

T^{ms} is an organic group detectable by mass spectrometry, comprising carbon, at least one of hydrogen and fluoride, and optional atoms selected from oxygen, nitrogen, sulfur, phosphorus and iodine;
L is an organic group which allows a unique T^{ms}-containing moiety to be cleaved from the remainder of the compound, wherein the T^{ms}-containing moiety comprises a functional group which supports a single ionized charge state when the compound is subjected to mass spectrometry and is tertiary amine, quaternary amine or organic acid; and
X' is nucleic acid.

24. A method for determining the quantity of a single nucleotide polymorphism in a nucleic acid target comprising:

a) amplifying a sequence of a nucleic acid target containing a single nucleotide polymorphism;
b) generating a single strand form of the target;
c) combining a tagged nucleic acid probe with the amplified target nucleic acid molecules under conditions and for a time sufficient to permit hybridization of said tagged nucleic acid probe to complementary amplified selected target nucleic acid molecules, wherein said tag is correlative with a particular single nucleotide polymorphism and is detectable by spectrometry or potentiometry;
d) separating unhybridized tagged probe from hybridized tagged probe by a sizing methodology;
e) cleaving said tag from said probes;
f) detecting said tags by spectrometry or potentiometry; and
j) taking the ratio of tag correlated to the wild type polymorphism to the tag correlated with the mutant polymorphism, and determining therefrom the quantity of said polymorphism,

wherein the tagged nucleic acid has the structure

$$T^{ms}\text{-}L\text{-}X'$$

wherein,

T^{ms} is an organic group detectable by mass spectrometry, comprising carbon, at least one of hydrogen and fluoride, and optional atoms selected from oxygen, nitrogen, sulfur, phosphorus and iodine;
L is an organic group which allows a unique T^{ms}-containing moiety to be cleaved from the remainder of the compound, wherein the T^{ms}-containing moiety comprises a functional group which supports a single ionized charge state when the compound is subjected to mass spectrometry and is tertiary amine, quaternary amine or organic acid; and
X' is nucleic acid.

Revendications

1. Composé de formule :

$$T^{ms}\text{-}L\text{-}X$$